CLAIMS

1. A method of detecting the presence of a target nucleic acid molecule comprising:

a) providing:

i) a cleavage means,

ii) a source of a first target nucleic acid, said first target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said first oligonucleotide contains a sequence complementary to said second region of said first target nucleic acid and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said first target nucleic acid;

iv) a second oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said first region of said first target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said second region of said first target nucleic acid;

v) a source of a second target nucleic acid, said second target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

vi) a third oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said second region of said second target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said third region of said second target nucleic acid;

b) generating a first cleavage structure wherein at least said 3' portion of said first oligonucleotide is annealed to said first target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said first target nucleic acid and wherein cleavage of said first cleavage structure occurs via said cleavage means thereby cleaving said first oligonucleotide to generate a fourth oligonucleotide, said fourth oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said fourth oligonucleotide contains a sequence complementary to said first region of said second target nucleic acid and wherein said 3' portion of said fourth oligonucleotide contains a sequence complementary to said second region of said second target nucleic acid;

c) generating a second cleavage structure under conditions wherein said at least said 3' portion of said third oligonucleotide is annealed to said second target nucleic acid and wherein at least said 5' portion of said fourth oligonucleotide is annealed to said second target nucleic acid oligonucleotide and wherein cleavage of said second cleavage structure occurs to generate a fifth oligonucleotide, said fifth oligonucleotide having a 3'-hydroxyl group; and

d) detecting said fifth oligonucleotide.

2. The method of Claim 1, wherein said first oligonucleotide has a length between eleven and fifteen nucleotides.

3. The method of Claim 1, wherein said cleavage means is a structure-specific nuclease.

4. The method of Claim 3, wherein said structure-specific nuclease is a thermostable structure-specific nuclease.

5. The method of Claim 4, wherein said thermostable structure-specific nuclease is a *Pyrococcus woesii* FEN-1 endonuclease.

6. The method of Claim 1, wherein one or more of said first, second, and said third oligonucleotides contain a dideoxynucleotide at the 3' terminus.

7. The method of Claim 1, wherein said detecting said fifth oligonucleotide comprises:

a) incubating said fifth oligonucleotide with a template-independent polymerase and at least one labelled nucleoside triphosphate under conditions such that at least one labelled nucleotide is added to the 3'-hydroxyl group of said fifth olignucleotide to generate a labelled fifth oligonucleotide; and

b) detecting the presence of said labelled fifth oligonucleotide.

8. The method of Claim 7, wherein said template-independent polymerase is selected from the group consisting of terminal deoxynucleotidyl transferase and poly A polymerase.

9. The method of Claim 8, wherein said third oligonucleotide contains a 5' end label, said 5' end label being a different label than the label present upon said labelled nucleoside triphosphate.

10. The method of Claim 1, wherein said detecting said fifth oligonucleotide comprises:

a) incubating said fifth oligonucleotide with a template-independent polymerase and at least one nucleoside triphosphate under conditions such that at least one nucleotide is added to the 3'-hydroxyl group of said fifth oligonucleotide to generate a tailed oligonucleotide; and

b) detecting the presence of said tailed fifth oligonucleotide.

11. The method of Claim 10, wherein said template-independent polymerase is selected from the group consisting of terminal deoxynucleotidyl transferase and poly A polymerase.

12. The method of Claim 11, wherein said third oligonucleotide contains a 5' end label.

13. The method of Claim 1, wherein said detecting said fifth oligonucleotide comprises:

a) providing:

i) said fifth oligonucleotide;

ii) a composition comprising two single-stranded nucleic acids annealed so as to define a single-stranded portion of a protein binding region;

iii) a nucleic acid producing protein;

b) exposing said fifth oligonucleotide to said single-stranded portion of said protein binding region under conditions such that said nucleic acid producing protein binds to said protein binding region and produces nucleic acid.

14. The method of Claim 13, wherein said single-stranded portion of said protein binding region comprises:

a) a first single continuous strand of nucleic acid comprising a sequence defining the template strand of an RNA polymerase binding region; and

b) a second single continuous strand of nucleic acid having a 5' and a 3' end, said second nucleic acid comprising a region complementary to a portion of said first nucleic acid, wherein said second nucleic acid is annealed to said first nucleic acid so as to define said single-stranded portion of said protein binding region.

15. The method of Claim 14, wherein said protein binding region is a template-dependent RNA polymerase binding region.

16. The method of Claim 15, wherein said template-dependent RNA polymerase binding region is the T7 RNA polymerase binding region.

17. The method of Claim 1, wherein said detecting said fifth oligonucleotide comprises:

a) providing:

i) said fifth oligonucleotide;

ii) a single continuous strand of nucleic acid comprising a sequence defining a single strand of an RNA polymerase binding region;

iii) a template-dependent DNA polymerase;

iv) a template-dependent RNA polymerase;

b) exposing said fifth oligonucleotide to said RNA polymerase binding region under conditions such that said fifth oligonucleotide binds to a portion of said single strand of said RNA polymerase binding region;

c) exposing said bound fifth oligonucleotide to said template-dependent DNA polymerase under conditions such that a double-stranded RNA polymerase binding region is produced; and

d) exposing said double-stranded RNA polymerase binding region to said template-dependent RNA polymerase under conditions such that RNA transcripts are produced.

18. The method of Claim 17 further comprising detecting said RNA transcripts.

19. The method of Claim 17, wherein said template-dependent RNA polymerase is the T7 RNA polymerase.

20. A method of detecting the presence of a target nucleic acid molecule comprising:

a) providing:

i) a cleavage means,

ii) a source of a first target nucleic acid, said first target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;

iii) a first oligonucleotide complementary to said fourth region of said first target nucleic acid;

iv) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said first target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said first target nucleic acid;

iv) a third oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said first target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said first target nucleic acid;

v) a source of a second target nucleic acid, said second target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

vi) a fourth oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said fourth oligonucleotide contains a sequence complementary to said second region of said second target nucleic acid and wherein said 3' portion of said fourth oligonucleotide contains a sequence complementary to said third region of said second target nucleic acid;

b) generating a first cleavage structure wherein said first oligonucleotide is annealed to said fourth region of said first target nucleic acid and wherein at least said 3' portion of said second oligonucleotide is annealed to said first target nucleic acid and wherein at least said 5' portion of said third oligonucleotide is annealed to said first target nucleic acid and wherein cleavage of said first cleavage structure occurs thereby cleaving said second oligonucleotide to generate a fifth oligonucleotide, said fifth oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said fifth oligonucleotide contains a sequence complementary to said first region of said second target nucleic acid and wherein said 3' portion of said fifth oligonucleotide contains a sequence complementary to said second region of said second target nucleic acid;

c) generating a second cleavage structure under conditions wherein said at least said 3' portion of said fourth oligonucleotide is annealed to said second target nucleic acid and wherein at least said 5' portion of said fifth oligonucleotide is annealed to said second target nucleic acid and wherein cleavage of said second cleavage structure occurs to generate a sixth oligonucleotide, said sixth oligonucleotide having a 3'-hydroxyl group; and

d) detecting said sixth oligonucleotide.

21. A method of detecting the presence of a target nucleic acid molecule comprising:

a) providing:

i) a cleavage means,

ii) a source of a target nucleic acid, said target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said first oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 5' portion of said first oligonuceotide contains a region of self-complementarity and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

iv) a second oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

v) a third oligonucleotide having a 5' and a 3' portion wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said 5' portion of said first oligonucleotide;

b) generating a first cleavage structure wherein at least said 3' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said target nucleic acid and wherein cleavage of said first cleavage structure

occurs thereby cleaving said first oligonucleotide to generate a fourth oligonucleotide, said fourth oligonucleotide having a first region, a second region and a third region, wherein said first region is located adjacent to and upstream of said second region and wherein said second region is located adjacent to and upstream of said third region and wherein said third region of said fourth oligonucleotide contains a a region of self-complementarity;

c) generating a second cleavage structure under conditions wherein said at least said 3' portion of said third oligonucleotide is annealed to said first region of said fourth oligonucleotide and wherein at least said 5' portion of said fourth oligonucleotide is annealed to said second region of said third olignucleotide and wherein said third region of said fourth oligonucleotide forms a hairpin structure and wherein cleavage of said second cleavage structure occurs to generate a fifth oligonuceotide, said fifth oligonucleotide having a 3'-hydroxyl group; and

d) detecting said fifth oligonucleotide.

22. A method of detecting the presence of human cytomegalovirus nucleic acid in a sample comprising:

a) providing:

i) a cleavage means,

ii) a sample suspected of containing human cytomegalovirus target nucleic acid, said target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said first oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

iv) a second oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

b) generating a cleavage structure wherein at least said 3' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said target nucleic acid and wherein cleavage of said cleavage structure occurs via said cleavage means to generate non-target cleavage products, each non-target cleavage product having a 3' hydroxyl group; and

c) detecting said non-target cleavage products and thereby detecting the presence of human cytomegalovirus nucleic acid in said sample.

23. The method of Claim 22, wherein said first oligonucleotide has a length between eleven and fifteen nucleotides.

24. The method of Claim 22, wherein said cleavage means is a thermostable structure-specific nuclease.

25. The method of Claim 24, wherein said nuclease is a *Pyroçoccus woesii* FEN-1 endonuclease.

26. The method of Claim 22, wherein one or more of said first and said second oligonucleotides contain a dideoxynucleotide at the 3' terminus.

27. The method of Claim 22, wherein said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one labelled nucleoside triphosphate under conditions such that at least one labelled nucleotide is added to the 3'-hydroxyl group of said non-target cleavage products to generate labelled non-target cleavage products; and

b) detecting the presence of said labelled non-target cleavage products.

28. The method of Claim 22, wherein said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one nucleoside triphosphate under conditions such that at least one nucleotide is added to the 3'-hydroxyl group of said non-target cleavage products to generate tailed non-target cleavage products; and

b) detecting the presence of said tailed non-target cleavage products.

29. The method of Claim 22, wherein said detecting said non-target cleavage products comprises:

a) providing:

i) said non-target cleavage products;

ii) a composition comprising two single-stranded nucleic acids annealed so as to define a single-stranded portion of a protein binding region;

iii) a nucleic acid producing protein;

b) exposing said non-target cleavage products to said single-stranded portion of said protein binding region under conditions such that said nucleic acid producing protein binds to said protein binding region and produces nucleic acid.

30. The method of Claim 29, wherein said single-stranded portion of said protein binding region comprises:

a) a first single continuous strand of nucleic acid comprising a sequence defining the template strand of an RNA polymerase binding region; and

b) a second single continuous strand of nucleic acid having a 5' and a 3' end, said second nucleic acid comprising a region complementary to a portion of said first nucleic acid, wherein said second nucleic acid is annealed to said first nucleic acid so as to define said single-stranded portion of said protein binding region.

31. The method of Claim 29, wherein said protein binding region is a template-dependent RNA polymerase binding region.

32. The method of Claim 31, wherein said template-dependent RNA polymerase binding region is the T7 RNA polymerase binding region.

33. The method of Claim 22, wherein said detecting said non-target cleavage products comprises:

a) providing:

i) said non-target cleavage products;

ii) a single continuous strand of nucleic acid comprising a sequence defining a single strand of an RNA polymerase binding region;

iii) a template-dependent DNA polymerase;

iv) a template-dependent RNA polymerase;

b) exposing said non-target cleavage products to said RNA polymerase binding region under conditions such that said non-target cleavage product binds to a portion of said single strand of said RNA polymerase binding region;

c) exposing said bound non-target cleavage products to said template-dependent DNA polymerase under conditions such that a double-stranded RNA polymerase binding region is produced;

d) exposing said double-stranded RNA polymerase binding region to said template-dependent RNA polymerase under conditions such that RNA transcripts are produced.

34. A method of detecting the presence of human cytomegalovirus nucleic acid in a sample comprising:

a) providing:

i) a cleavage means,

ii) a sample suspected of containing human cytomegalovirus target nucleic acid, said target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;

iii) a first oligonucleotide complementary to said fourth region of said target nucleic acid;

iv) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

v) a third oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

b) generating a cleavage structure wherein said first oligonucleotide is annealed to said fourth region of said target nucleic acid and wherein at least said 3' portion of said second oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said third oligonucleotide is annealed to said target nucleic acid and wherein cleavage of said cleavage structure occurs via said cleavage means to generate non-target cleavage products, each non-target cleavage product having a 3' hydroxyl group; and

c) detecting said non-target cleavage products and thereby detecting the presence of human cytomegalovirus nucleic acid in said sample.